International Preliminary Examining Authority
The European Patent Office
D-80298 Munich
GERMANY

15 December 2003

BY FACSIMILE

Dear Sirs

International Patent Application No PCT/EP02/14512
Applicant: Plant Bioscience Limited
Our Ref: SMK/LP6138390

I write in response to the Written Opinion dated 13 August 2003.

The examiner objected to claims 1-12 on grounds of lack of inventive step over D1 + D2. The examiner is requested to take the following comments into account and to re- consider the inventive step of the claimed subject matter when issuing the IPER.

The inventors on this application discovered that changing the distribution of ATP or ADP in a cell (for example by inhibiting a plastidial transporter of ATP/ADP) caused an increase in content of transgene-encoded biomolecules. For example, when the ATP/ADP transporter was inhibited in transgenic potatoes expressing scFv, a marked increase in scFv concentration was observed.

The improvements could be viewed as increasing the content of a transgene-coded biomolecule in an organism. The inventors achieved this by changing the cellular distribution of ATP or ADP in the organism.

The plastidial ATP/ADP transporter used by the inventors in the worked examples had previously been a focus of studies on starch synthesis in plants, because it exchanges ATP for ADP across the amyloplast wall to allow starch synthesis in the amyloplast (see pages 3-5 of the description, and Tjaden *et al.* cited in the ISR). Various effects on sugar-starch interconversions and on the activity of enzymes in this pathway were observed when expression of the transporter was altered.

So, the inventors have taken a transporter known to regulate starch synthesis in plants, and applied it in the context of increasing content of a transgene-coded biomolecule in an organism. No teaching in the prior art would have lead the skilled person to reasonably expect the content of transgene-coded biomolecules to be increased by altering ATP/ADP exchange.

The examiner refers to document D2, which reports the study of plants engineered to overexpress pyrophosphatase and consequently contain less pyrophosphate than normal plants. The D2 authors studied levels of the metabolites and enzyme activities involved in starch-sugar interconversion, and determined the levels of sugar and starch in the modified plants compared to wild-type. D2 reports that pyrophosphatase-overexpressing plants had altered levels of metabolites and activities of enzymes involved in the sugar/starch metabolism. Among other things, levels of ATP and ADP were higher in the pyrophosphatase-overexpressing plants than in wild-type.

D2 is concerned with plant carbohydrate metabolism, not transgene-coded biomolecule production. Thus, D2 is an unlikely source of guidance for the skilled person seeking to increase the content of a transgene-coded biomolecule in an organism.

Even if the skilled person did consider the teaching of D2 in relation to increasing transgene-coded biomolecule content, the changes observed in D2 provide no indication that the modified plants would show an increased content of a given transgene-coded biomolecule. The D2 authors expected the engineered decrease in pyrophosphate to cause changes in sugar-starch interconversion, and the observed changes are reported in D2. These changes, including altered enzyme activities and metabolite concentrations, are attributed to the influence of pyrophosphate and nucleotide pools on metabolic flux control of enzymes in carbohydrate metabolism pathways, especially sugar-starch interconversion, owing to the involvement of nucleotides in regulating these particular reactions. On the other hand, there would be no reason to expect changes in gene expression of a given transgene inserted into cells of these plants. There is no suggestion in D2 that gene expression is generally altered. The mere fact that levels of some biomolecules in carbohydrate metabolism are altered does not lead the skilled person to to expect that the level of a given transgene-coded biomolecule would also alter. Moreover, there would be no reason to reasonably expect an *increase* in content of a biomolecule, rather than a decrease.

Furthermore, even if the D2 plants did show an increased content of a given transgene-coded biomolecule (which they do not), the skilled person would not be able to infer that it was the result of a change in ATP or ADP. The changes in ATP and ADP observed in the D2 plants were just one of a number of effects caused by overexpression of pyrophosphatase and decrease in pyrophosphate. The examiner stated that a change in ATP influenced enzyme activities and the content of various compounds, but this is an over-simplification. The changes resulted from the depletion of pyrophosphate in the cells and from the consequent shift in metabolic control of the enzyme-catalysed pathways. The level of ATP was changed, but the effects of this change cannot be separated in D2 from the related effects of changes to other metabolites.

Finally, for completeness we note that whereas the present invention is concerned with changing the ATP or ADP <u>distribution</u> in a cell, D2 merely reports an increase in ATP and ADP and is silent about their distribution.

In summary, neither D1 nor D2 nor the combination thereof gives the skilled person no reasonable expectation of being able to increase the content of a transgene-coded biomolecule in an organism by changing the distribution of ATP or ADP in cells of the organism. The presently claimed subject matter therefore involves an inventive step over the cited art.

A favourable Preliminary Examination Report is kindly requested.

Yours faithfully

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